METHOD FOR TREATING OCCLUSIVE VASCULAR DISEASES & WOUND HEALING

FIELD OF THE INVENTION

Compositions and methods for treatment of wound healing, occlusive peripheral vascular, carotid and coronary disease are disclosed. The compositions and methods allow treatment of diseases associated with occlusion of coronary vessels, for example, by promoting growth of new blood vessels, i.e., angiogenesis and/or by recruitment of collaterals. The methods involve the administration of polymeric forms of Nicotinic acid derivatives or nicotine alone or in combination with other pro-angiogenesis agents and / or vasodilators over a period of several days. In particular, this invention is applicable to improving wound healing, collateral coronary, peripheral artery, and carotid circulation in patients suffering from impaired wound healing, impotence, myocardial infarction, peripheral artery diseases, and stroke.

BACKGROUND OF THE INVENTION

It is estimated that five million people are afflicted with chronic stable angina in the United States. Each year 200,000 people under the age of 65 die with what is termed "premature ischemic heart disease." Despite medical therapy, many go on to suffer myocardial infarction and debilitating symptoms prompting the need for revascularization with either percutaneous transluminal coronary angioplasty or coronary artery bypass surgery. Medical researchers have postulated that one way of relieving myocardial ischemia would be to enhance coronary collateral circulation.

Fujita et. al. (Fujita et al., Am. Heart Journal., 122:453 (1991), Fujita et al., Int. J. Cardiol., 40:51 (1993)) demonstrated that heparin in combination with short-term exercise training improved exercise tolerance as measured by dynamic exercise testing. The researchers, believing this effect was mediated through increased collateral vascular development, examined the effects of heparin in combination with a brief concomitant exercise training

protocol on coronary collateral flow. Thallium-201 myocardial perfusion images obtained in association with the same workload both before and late after combined heparin exercise treatment, which indicated that coronary collateral circulation was enhanced. Such dramatic changes over a short term do not occur naturally, and suggest that angiogenesis has taken place.

Correlations have now been made between the anatomic appearance of coronary collateral vessels ("collaterals") visualized at the time of intracoronary thrombolitic therapy during the acute phase of myocardial infarction and the creatine kinase time-activity curve, infarct size, and aneurysm formation. These studies demonstrate a protective role of collaterals in hearts with coronary obstructive disease, showing smaller infarcts, less aneurysm formation, and improved ventricular function compared with patients in whom collaterals were not visualized.

When the cardiac myocyte is rendered ischemic, collaterals develop actively by growth with DNA replication and mitosis of endothelial and smooth muscle cells. One hypothesis suggests that heparin-binding growth factors are present in the heart, or that biological activity is quiescent under normal physiological conditions. Once ischemia develops, these factors are activated and become available for receptor occupation, which may initiate angiogenesis after exposure to exogenous heparin. Unfortunately, the "natural" process by which angiogenesis occurs is inadequate to reverse the ischemia in almost all patients with coronary artery disease.

During ischemia, adenosine is released through the breakdown of ATP. Adenosine participates in many cardio-protective biological events. Adenosine has a role in hemodynamic changes such as bradycardia and vasodilation, and adenosine has been suggested to have a role in such unrelated phenomena as preconditioning and possibly the reduction in reperfusion injury (Ely and Beme, Circulation, 85: 893 (1992).

Intrinsic adenosine may facilitate the coronary flow response to increased myocardial oxygen demands and so modulate the coronary flow reserve. Ethier et. al. (Ethier et al., Am. J. Physiol., H131 (1993)) demonstrated that the addition of physiological concentrations of adenosine to human umbilical vein endothelial cell cultures stimulates proliferation, possibly

via a surface receptor. They suggested that adenosine may be a factor for human endothelial cell growth and possibly angiogenesis. Angiogenesis appears to be protective for patients with CAD, but the rate at which blood vessels grow naturally is inadequate to reverse the disease. Thus, strategies to enhance and accelerate the body's natural angiogenesis potential should be beneficial in patients with CAD.

Over 150 million men worldwide suffer from erectable dysfunction and only a small percentage is being treated for it. Although a number of diseases such as diabetes can be the cause, in most cases the underlying problem can't be identified. Viagra was the first oral drug to be approved by the U.S. Food and Drug Administration for erectility dysfunction. Since its approval, over 17 million men have received Viagra worldwide. Other manufacturers are rushing products through clinical trials to compete with Viagra. Viagra, Levitra and Cialis all work to reduce the effects of an enzyme called PDE5. Reducing the activity of the PDE5 enzyme means more blood can flow to the penis and less leaves. A combined use of thyroid hormone analogs topically or systemically with hormonally inactive analogs that sustain potent pro-angiogenesis effects would be of value in enhancing the effects of other standard therapies such as listed above, vasodilators, and others.

There remains a need for an effective therapy for promotion of coronary angiogenesis with minimum side effects. Such a therapy would be particularly useful for patients who have myocardial infarctions and could be used prophylactically in patients who have poor coronary circulation, which places them at high risk of ischemia and myocardial infarctions.

Nicotine stimulates new blood vessel growth: Most forms of smoking cessation treatment involve the use of nicotine without tobacco. Thus, the effects of such doses of nicotine on the body are crucial. However, according to new research published in the July issue of (Nature Medicine, Vol. 7, July 2001, page 833), the bad news for those using these products to stop smoking is that nicotine without tobacco can cause angiogenesis, which in turn aids the growth of atherosclerotic plaques and tumors.

We have made the startling observation that either nicotine or nicotinic acid (Vitamin B3) has potent angiogenic properties. The angiogenic effects of nicotine but not nicotinic acid are mediated in part by an endothelial nicotinergic receptor, the vitronectin receptors and the release of endothelial FGF2. The angiogenic effects of nicotinic acid and derivatives are mediated in part by the release of endothelial FGF2.

SUMMARY OF THE INVENTION

Compositions and methods for treatment of occlusive peripheral vascular disease and coronary diseases, in particular, the occlusion of coronary vessels, and disorders associated with the occlusion of the peripheral vasculature and/or coronary blood vessels, are disclosed. Also disclosed are compositions and methods for promoting angiogenesis and/or recruiting collateral blood vessels in a patient in need thereof. The compositions include an effective amount of polymeric forms of nicotinic acid and nicotine and an effective amount of an adenosine and / or nitric oxide donor. The compositions can be in the form of a sterile, injectable, pharmaceutical formulation that includes an angiogenically effective amount of nicotinic acid and nicotine-like substance and adenosine derivatives in a physiologically and pharmaceutically acceptable carrier, optionally with one or more excipients.

The methods involve the co-administration of an effective amount of nicotinic acid and nicotine-like substance and an effective amount of an adenosine and / or NO donor in low, daily dosages for a week or more. One or both components can be delivered locally via catheter. Nicotinic acid or nicotine, and derivatives in vivo can be delivered to capillary beds surrounding ischemic tissue by incorporation of the compounds in an appropriately sized liposome or microparticle. Nicotinic acid or nicotine, polymeric forms and derivatives can be targeted to ischemic tissue by covalent linkage with a suitable antibody.

The method may be used as a treatment to restore cardiac function after a myocardial infarction. The method may also be used to improve blood flow in patients with coronary artery disease suffering from myocardial ischemia or inadequate blood flow to areas other than

the heart, for example, occlusive peripheral vascular disease (also known as peripheral arterial occlusive disease), where decreased blood flow is a problem.

DETAILED DESCRIPTION OF THE INVENTION

Compositions and methods for treatment of occlusive peripheral vascular disease and coronary diseases, in particular, the occlusion of coronary vessels, and disorders associated with the occlusion of the peripheral vasculature and/or coronary blood vessels are disclosed. Also disclosed are compositions and methods for promoting angiogenesis and/or recruiting collateral blood vessels in a patient in need thereof. The compositions include an effective amount of Nicotinic acid or nicotine, polymeric forms, and derivatives. The methods involve the co-administration of an effective amount of Nicotinic acid or nicotine, polymeric forms, and derivatives in low, daily dosages for a week or more.

Definitions

As used herein, the term "myocardial ischemia" is defined as an insufficient blood supply to the heart muscle caused by a decreased capacity of the heart vessels.
As used herein, the term "coronary disease" is defined as diseases/disorders of cardiac function due to an imbalance between myocardial function and the capacity of coronary vessels to supply sufficient blood flow for normal function. Specific coronary diseases/disorders associated with coronary disease which can be treated with the compositions and methods described herein include myocardial ischemia, angina pectoris, coronary aneurysm, coronary thrombosis, coronary vasospasm, coronary artery disease, coronary heart disease, coronary occlusion and coronary stenosis.

As used herein the term "occlusive peripheral vascular disease" (also known as peripheral arterial occlusive disorder) is a vascular disorder-involving blockage in the carotid or femoral arteries, including the iliac artery. Blockage in the femoral arteries causes pain and restricted movement. A specific disorder associated with occlusive peripheral vascular disease is diabetic foot, which affects diabetic patients, often resulting in amputation of the foot.

As used herein the terms "regeneration of blood vessels," angiogenesis," "revascularization," and "increased collateral circulation" (or words to that effect) are considered as synonymous. The term "pharmaceutically acceptable" when referring to a natural or synthetic substance means that the substance has an acceptable toxic effect in view of its much greater beneficial effect, while the related the term, "physiologically acceptable," means the substance has relatively low toxicity. The term, "co-administered" means two or more drugs are given to a patient at approximately the same time or in close sequence so that their effects run approximately concurrently or substantially overlap. This term includes sequential as well as simultaneous drug administration.

"Pharmaceutically acceptable salts" refers to pharmaceutically acceptable salts of Nicotinic acid or nicotine, polymeric forms, and derivatives, which salts are derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetra-alkyl ammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the like can be used as the pharmaceutically acceptable salt.

METHODS OF TREATMENT

Nicotinic acid or nicotine, polymeric forms, and derivatives can be used in a method for promoting angiogenesis in a patient in need thereof. The method involves the co-administration of an effective amount of Nicotinic acid or nicotine, polymeric forms, and derivatives in low, daily dosages for a week or more. The method may be used as a treatment to restore cardiac function after a myocardial infarction. The method may also be used to improve blood flow in patients with coronary artery disease suffering from myocardial ischemia or inadequate blood flow to areas other than the heart, for example, peripheral vascular disease, for example, peripheral arterial occlusive disease, where decreased blood flow is a problem.

The compounds can be administered via any medically acceptable means which is suitable for the compound to be administered, including oral, rectal, topical or parenteral (including

subcutaneous, intramuscular and intravenous) administration. For example, adenosine has a very short half-life. For this reason, it is preferably administered intravenously. However, adenosine A.sub.2 agonists have been developed which have much longer half-lives, and which can be administered through other means. Nicotinic acid or nicotine, polymeric forms, and derivatives can be administered, for example, intravenously, oral, topical, intranasal administration.

In some embodiments, the Nicotinic acid or nicotine, polymeric forms, and derivatives via different means of administration. The amounts of the Nicotinic acid or nicotine, polymeric forms, and derivatives required to be effective in stimulating angiogenesis will, of course, vary with the individual being treated and is ultimately at the discretion of the physician. The factors to be considered include the condition of the patient being treated, the efficacy of the particular adenosine A.sub.2 receptor agonist being used, the nature of the formulation, and the patient's body weight. Occlusion-treating dosages of Nicotinic acid or nicotine, polymeric forms, and derivatives are any dosages that provide the desired effect.

FORMULATIONS

The compounds described above are preferably administered in a formulation including Nicotinic acid or nicotine, polymeric forms, and derivatives together with an acceptable carrier for the mode of administration. Any formulation or drug delivery system containing the active ingredients, which is suitable for the intended use, as are generally known to those of skill in the art, can be used. Suitable pharmaceutically acceptable carriers for oral, rectal, topical or parenteral (including subcutaneous, intraperitoneal, intramuscular and intravenous) administration are known to those of skill in the art. The carrier must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Formulations suitable for parenteral administration conveniently include sterile aqueous preparation of the active compound, which is preferably isotonic with the blood of the recipient. Thus, such formulations may conveniently contain distilled water, 5% dextrose in distilled water or saline. Useful formulations also include concentrated solutions or solids

containing the compound of formula (I), which upon dilution with an appropriate solvent give a solution suitable for parental administration above.

For enteral administration, a compound can be incorporated into an inert carrier in discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the active compound; as a powder or granules; or a suspension or solution in an aqueous liquid or non-aqueous liquid, e.g., a syrup, an elixir, an emulsion or a draught. Suitable carriers may be starches or sugars and include lubricants, flavorings, binders, and other materials of the same nature.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form, e.g., a powder or granules, optionally mixed with accessory ingredients, e.g., binders, lubricants, inert diluents, surface active or dispersing agents. Molding in a suitable machine, a mixture of the powdered active compound may make molded tablets with any suitable carrier.

Adding the active compound to a concentrated, aqueous solution of a sugar, e.g., sucrose, to which may also be added any accessory ingredients, may make a syrup or suspension. Such accessory ingredients may include flavoring, an agent to retard crystallization of the sugar or an agent to increase the solubility of any other ingredient, e.g., as a polyhydric alcohol, for example, glycerol or sorbitol.

Formulations for rectal administration may be presented as a suppository with a conventional carrier, e.g., cocoa butter or Witepsol S55 (trademark of Dynamite Nobel Chemical, Germany), for a suppository base.

Alternatively, the compound may be administered in liposomes or microspheres (or microparticles). Methods for preparing liposomes and microspheres for administration to a patient are well known to those of skill in the art. U.S. Pat. No. 4,789,734, the contents of which are hereby incorporated by reference, describes methods for encapsulating biological

materials in liposomes. Essentially, the material is dissolved in an aqueous solution, the appropriate phospholipids and lipids added, along with surfactants if required, and the material dialyzed or sonicated, as necessary. A review of known methods is provided by G. Gregoriadis, Chapter 14, "Liposomes," Drug Carriers in Biology and Medicine, pp. 287-341 (Academic Press, 1979).

Microspheres formed of polymers or proteins are well known to those skilled in the art, and can be tailored for passage through the gastrointestinal tract directly into the blood stream. Alternatively, the compound can be incorporated and the microspheres, or composite of microspheres, implanted for slow release over a period of time ranging from days to months. See, for example, U.S. Pat. Nos. 4,906,474, 4,925,673 and 3,625,214, and Jein, TIPS 19:155-157 (1998), the contents of which are hereby incorporated by reference.

In one embodiment, the Nicotinic acid or nicotine, polymeric forms, and adenosine derivatives can be formulated into a liposome or microparticle, which is suitably sized to lodge in capillary beds following intravenous administration. When the liposome or microparticle is lodged in the capillary beds surrounding ischemic tissue, the agents can be administered locally to the site at which they can be most effective. Suitable liposomes for targeting ischemic tissue are generally less than about 200 nanometers and are also typically unilamellar vesicles, as disclosed, for example, in U.S. Pat. No. 5,593,688 to Baldeschweiler, entitled "Liposomal targeting of ischemic tissue," the contents of which are hereby incorporated by reference.

Preferred microparticles are those prepared from biodegradable polymers, such as polyglycolide, polylactide and copolymers thereof. Those of skill in the art can readily determine an appropriate carrier system depending on various factors, including the desired rate of drug release and the desired dosage.

In one embodiment, the formulations are administered via catheter directly to the inside of blood vessels. The administration can occur, for example, through holes in the catheter. In those embodiments wherein the active compounds have a relatively long half life (on the order of 1 day to a week or more), the formulations can be included in biodegradable polymeric

hydrogels, such as those disclosed in U.S. Pat. No. 5,410,016 to Hubbell et al. These polymeric hydrogels can be delivered to the inside of a tissue lumen and the active compounds released over time as the polymer degrades. If desirable, the polymeric hydrogels can have microparticles or liposomes which include the active compound dispersed therein, providing another mechanism for the controlled release of the active compounds.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active compound into association with a carrier, which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier or a finely divided solid carrier and then, if necessary, shaping the product into desired unit dosage form.

The formulations can optionally include additional components, such as various biologically active substances such as growth factors (including TGF-.beta, basic fibroblast growth factor (FGF2), epithelial growth factor (EGF), transforming growth factor (TGF alpha and beta), nerve growth factor (NGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor/vascular permeability factor (VEGF/VPF)), antiviral, antibacterial, anti-inflammatory, immunosuppressant, analgesic, vascularizing agent, and cell adhesion molecule.

In addition to the aforementioned ingredients, the formulations may further include one or more optional accessory ingredient(s) utilized in the art of pharmaceutical formulations, e.g., diluents, buffers, flavoring agents, binders, surface active agents, thickeners, lubricants, suspending agents, preservatives (including antioxidants) and the like.

MATERIALS & METHODS

Reagents: All reagents were chemical grade and purchased from Sigma Chemical Co. (St. Louis, MO) or through VWR Scientific (Bridgeport, NJ). Cortisone acetate, bovine serum albumin (BSA) and gelatin solution (2% type B from bovine skin) were purchased from Sigma

Chemical Co. Fertilized chicken eggs were purchased from Charles River Laboratories, SPAFAS Avian Products & Services (North Franklin, CT).

Chorioallantoic membrane (CAM) assay: Microscopic Analysis of CAM Sections

In vivo neovascularization was examined by the method previously described by Auerbach et al. (J. Dev. Biol. 41:391-394 (1974)). Ten-day old embryos were purchased from Spafas, Inc. (Preston, CT) and were incubated at 37 °C with 55% relative humidity. In the dark with the help of a candling lamp a small hole was punctured in the shell concealing the air sac with a hypodermic needle. A second hole was punctured in the shell on the broadside of the egg directly over a vascular portion of the embryonic membrane, as observed during candling. A false air sac was created beneath the second hole by the application of negative pressure to the first hole, which caused the chorioallantoic membrane (CAM) to separate from the shell. A window, approximately 1.0 cm^2, was cut in the shell over the dropped CAM with the use of a small crafts grinding wheel (Dremel, Division of Emerson Electric Company Racine, Wisconsin), which allowed direct access to the underlying CAM. Filter disks of #1 filter paper (Whatman International, United Kingdom) were soaked in 3 mg/mL cortisone acetate (Sigma, St. Louis, MO) in a solution of 95% ethanol and water and subsequently air dried under sterile conditions. FGF2 (Life Technologies, Gaithersburg, Maryland) was used as standard to grow vessels on the CAM of 10-d old chick embryos. Nicotine, Nicotinic acid, and derivatives were compared to FGF2. Sterile filter disks adsorbed with FGF2 or thyroid hormone analogs were dissolved in PBS at 1μg/ml were placed on growing CAM. At 24 h, test compounds or control vehicle was added directly to CAM topically.

CAM tissue directly beneath FGF2-saturated filter disk was resected from embryos treated 48 h prior with compound or control. Tissues were washed three times with PBS. Sections were placed in a 35-mm petri dish (Nalge Nunc, Rochester, New York) and examined under a SV6 stereomicroscope (Karl Zeiss, Thornwood, New York) at 50X magnification. Digital images of CAM sections adjacent to filters were collected using a 3-CCD color video camera system (Toshiba America, New York, NY) and analyzed with the Image-Pro Plus software (Media Cybernetics, Silver Spring, MD)

Effect of Nicotine, Nicotinic acid, and derivatives on angiogenesis.

Nicotine, Nicotinic acid, and ppolymeric conjugates induced significant increase in angiogenesis index (fold increase above basal) in the CAM model (Table 1).

Enhancement of pro-angiogenic activity of FGF2 by sub-maximal concentrations of T_4.

The combination of Nicotine and FGF2 at sub-maximal concentrations resulted in an additive increase in the angiogenesis index up to the same level like the maximal pro-angiogenesis effect of either FGF2 or Nicotine and Nicotinic acid (Table 2).

EXAMPLES

EXAMPLE 1: Angiogenesis in the CAM model. FGF2 versus T4 or T4-garose

Thyroid hormone analogs produced comparable pro-angiogenesis effect to that observed with standard pro-angiogenic growth factors such as FGF2 in the CAM model (Table 1, Figure 1).

TABLE 1: Pro-angiogenesis effect of FGF2, Nicotine ot Nicotinic acid polymeric form in the CAM Model

Treatment	Mean Number of Branch Points ± SD
Control	76 ± 10
FGF2 (1.0 μg)	195 ± 22**
Nicotine (0.1 μg)	192 + 11**
Nicotinic acid (1.0 μg)	155 ± 16*
Nicotinic acid polymeric form I (0.1 μg)	188 + 14**

*Data represent average branch point ±SD, n = 8, * P < 0.01, ** P <0.001. Nicotinic acid polymer conjugated through an ester linkage with polyvinyl alcohol.*

EXAMPLE 2: Nicotine, Nicotinic acid and FGF2

An additive pro-angiogenic effect was demonstrated when combining Nicotine and FGF2 at sub-maximal levels (Table 2).

Table 2: Additive Pro-angiogenesis effects of FGF2 and Nicotine on Angiogenesis index (number of branch points) in the CAM Model

Treatment	Average Number Of Branch Points
Control	86 ± 14
FGF2 (1.0 μg)	195 ± 22**
Nicotine (0.1 μg)	192 + 11**
FGF2 (0.5 μg)	115 ± 8*
Nicotine (0.05 μg)	145 ± 10*
FGF2 (0.5 μg) + Nicotine (0.05 μg)	225 ± 15**

*Data represent average branch points ± SD, n = 16, * P < 0.05, P < 0.001. Similar data were shown with Nicotinic acid or its polymeric form with FGF2.*

This invention provides novel compositions and methods for treatment of occlusive peripheral vascular disease and coronary diseases, in particular, the occlusion of coronary vessels, and disorders associated with the occlusion of the peripheral vasculature and/or coronary blood vessels, are disclosed. Also disclosed are compositions and methods for promoting angiogenesis and/or recruiting collateral blood vessels in a patient in need thereof. The compositions include an effective amount of polymeric forms of Nicotinic acid, analogs and derivatives, with an effective amount of an adenosine and / or nitric oxide donor o other vasodilators. The compositions can be in the form of a sterile, injectable, pharmaceutical formulation that includes an angiogenically effective amount of Nicotine, nicotinic acid,